Cell Biology

ELUCIDATING THE ROLE OF THE CYTOPLASMIC DOMAIN IN MECHANISMS OF TNF-ALPHA CONVERTING ENZYME (TACE) ACTIVATION

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Alzheimer's disease is characterized by the progressive formation of insoluble amyloid plaques in the brain. Cleavage of amyloid precursor protein (APP) by β- and γ-secretases generates amyloid-β (Aβ), the major component of senile plaques. Cleavage of APP by α-secretase prevents Aβ formation, producing nonamyloidogenic APP secretion products. Recent evidence has shown that a phorbol ester, PMA, affects protein kinase C (PKC) to upregulate α-secretase activity, leading to the premise that α-secretase activity and its phosphorylation state are in some way related. In order to substantiate the importance of the phosphorylation state, Chinese hamster ovary cells stably transfected with the 695 amino-acid isoform of APP (CHO695) were exposed to treatments of PMA in addition to varying concentrations of okadaic acid, a specific inhibitor of protein phosphatase(PP)-1 and PP-2A. Through several experiments employing Western Blot analysis of APP cleavage products secreted from CHO695 cells, we have been able to determine that phosphatase inhibition is another mechanism by which α-secretase activity is enhanced. Additional experiments have shown that PP-1 is the major phosphatase regulating α-secretases. Since the cytoplasmic domain of α-secretase is the likely site for interaction/regulation by PKC, and because TACE is a known α-secretase, we designed cytoplasmic tail mutations and tested their ability to induce α-cleavage of APP following transfection into TACE knockout fibroblasts. Results from this experiment were inconclusive, but further studies would give greater insight into the competition between α- and β-secretases for intracellular cleavage, which may represent a novel target for the discovery of new therapeutic agents to treat Alzheimer's disease.